

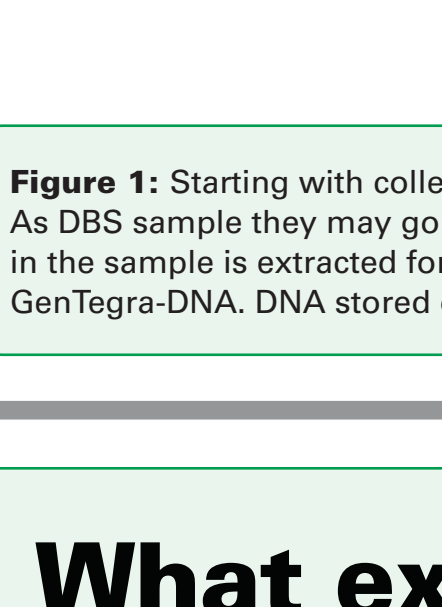
## Biobanking of Whole Blood and Nucleic Acid Samples at Ambient – A Green Solution for Biobanking

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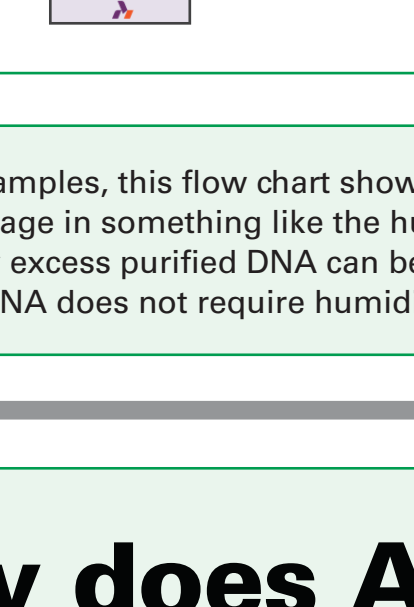
### Abstract

Sample biobanking becomes necessary when the specimens of interest are precious and used to support clinical trials, epidemiology and biomarker discovery. The Brain Initiative and the Precision Medicine initiatives are intended to collect a million cohort samples of that kind. Tissue biopsy samples have been banked effectively for decades as Formalin Fixed Paraffin Embedded samples. The other commonly banked tissue sample for genomics is EDTA blood, as it is pristine, minimally invasive and readily available donor sample. The common method for storage of such blood is by freezing at -80°C. The logistics of storage and retrieval of these frozen samples requires power backup in case of a catastrophe. Ambient (room temperature) storage is an attractive option. Storage at ambient temperature of such donor samples as Dried Blood Spots (DBS) is a paradigm shift from the norm but one which is independent of the power grid, has a 100-fold lower carbon footprint, is 100 times more compact and readily suited to automated recovery. Other than biobanking; DBS samples can be an effective and convenient means of collection of samples from participants in Clinical Trials. The trial candidate would be more compliant to sample collection in the comfort of their own home. Blood stabilized on the DBS can then be mailed by local postal services at their convenience. Although storage of whole blood as DBS is an old technology, the adoption of DBS has been hindered by low recoveries and low quality of the extracted nucleic acid. We present here a completely new paper-based technology to overcoming these drawbacks, deployed as an improved DBS collection card and matched nucleic acid extraction method that yields DNA with quality and quantity sufficient to support advanced methods such as Next Generation Sequencing.

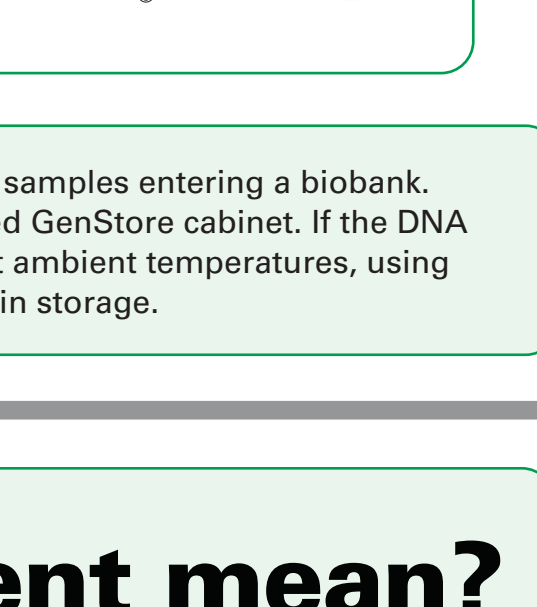
### Step 1: Sample Collection Cards & GenPlates™



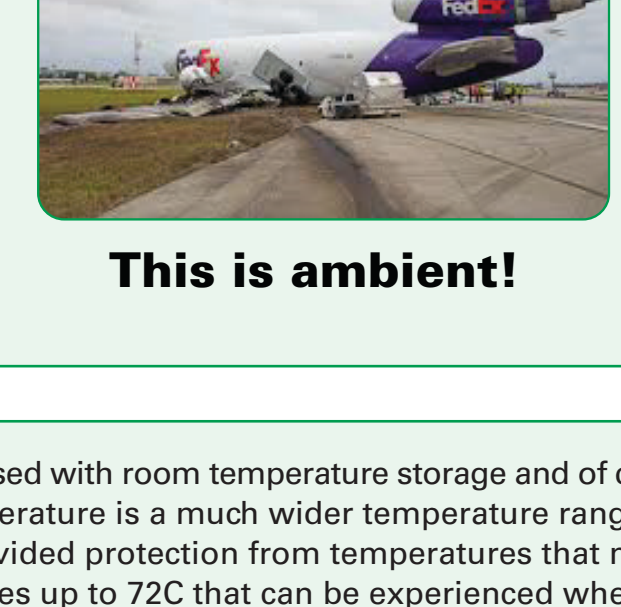
### Step 2: Sample Storage Humidity Controlled Cabinet



### Step 3: DNA Extraction & Purification

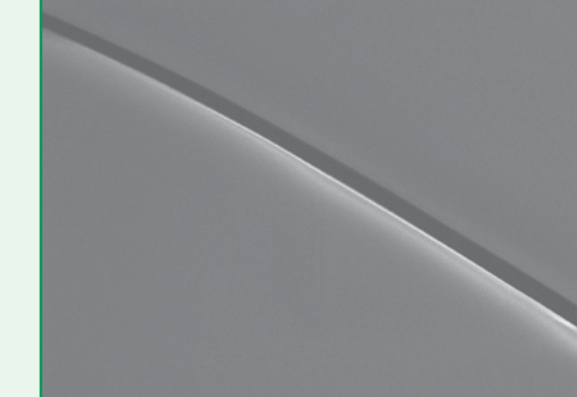


### Step 4: DNA Storage at Ambient on GenTegra-DNA

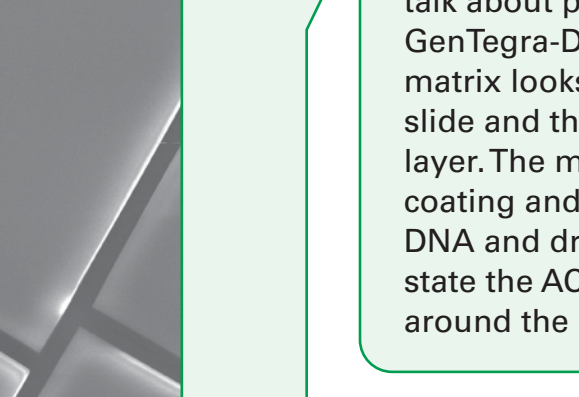


**Figure 1:** Starting with collection of DBS samples, this flow chart shows the process of samples entering a biobank. As DBS sample they may go directly to storage in something like the humidity controlled GenStore cabinet. If the DNA in the sample is extracted for NGS then any excess purified DNA can be stored, again at ambient temperatures, using GenTegra-DNA. DNA stored on GenTegra-DNA does not require humidity control while in storage.

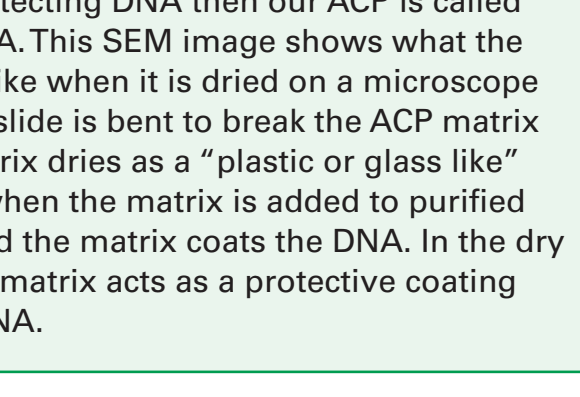
## What exactly does Ambient mean?



**This is ambient!**



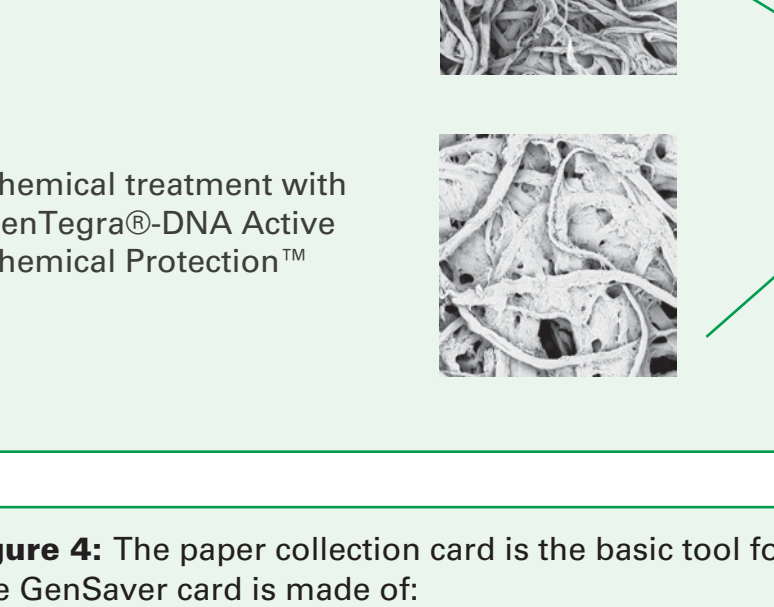
**This is ambient!**



**This is NOT ambient!**

## Active chemical protection™

### What it is & how it works



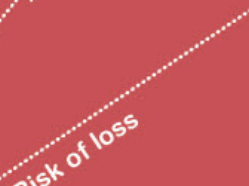
SEM Image of ACP/DNA complex

**Figure 3:** When planning stable ambient temperature storage of biological molecules, be it nucleic acids, proteins or any other molecules, some added form of protection is required to prevent damage that will occur if the samples are simply stored at ambient. GenTegra uses a chemical matrix called ACP, Active Chemical Protection. The matrix that provides this ACP differs depending upon the type of molecule being protected. If we talk about protecting DNA then our ACP is called GenTegra-DNA. This SEM image shows what the matrix looks like when it is dried on a microscope slide and the slide is bent to break the ACP matrix layer. The matrix dries as a "plastic or glass like" coating and when the matrix is added to purified DNA and dried the matrix coats the DNA. In the dry state the ACP matrix acts as a protective coating around the DNA.

## Development of GenSaver™ collection card

Ahlstrom-Munksjö GenSaver™ specimen collection cards allow long term preservation at ambient temperature of DNA in biological fluids.

Cotton fiber-based material with pure and absorbent fibers



Chemical treatment with GenTegra®-DNA Active Chemical Protection™

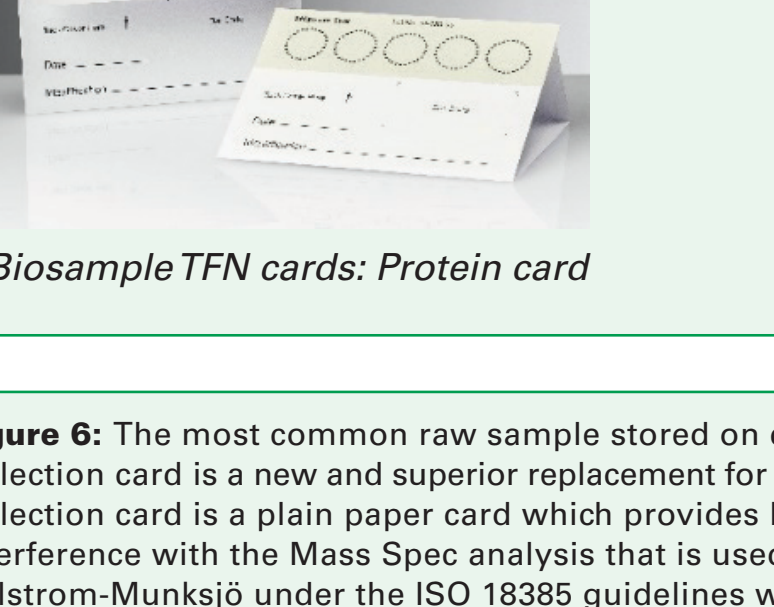


Maximum volume loaded/ sample area:  
Blood: 165 µl Saliva: 125 µl

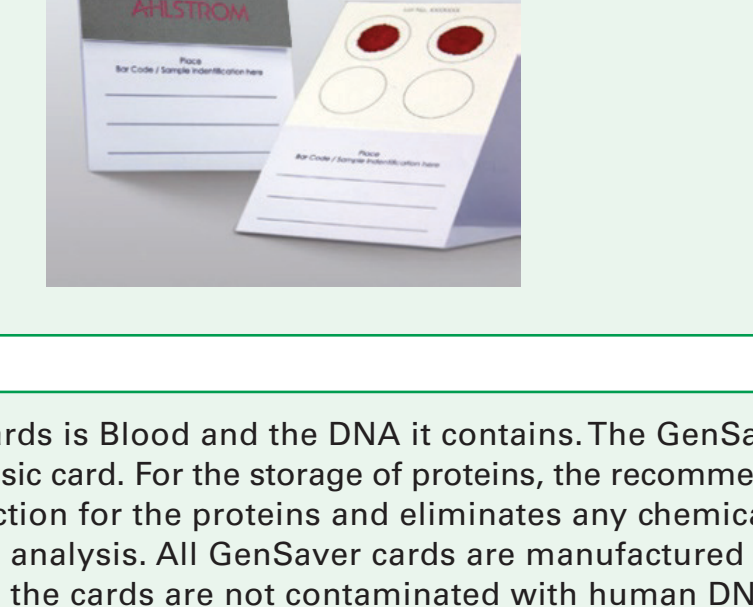
**Figure 4:** The paper collection card is the basic tool for ambient storage of raw DBS samples. The GenSaver card is made of:

- Pure cotton based absorbent fibers
- Impregnation with stabilizing chemistry, GenTegra-DNA Active Chemical Protection, to protect and stabilize the DNA in the sample.
- The common 4-spot collection card is shown with SEM images of both the plain paper and the same paper after the ACP matrix has been added. Thanks to the combination of fibers and chemistry, samples can be collected in remote locals and transport to the laboratory without the need for a cold chain.
- The GenSaver cards enable direct amplification for STR analysis even after the DBS has been stored for 20-years or more.

## Increased storage security



McMaster University freezer archive survey



Manhattan power outage during Hurricane Sandy

### Benefits of GenTegra's Ambient Storage Technology

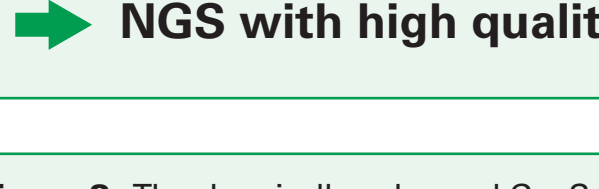
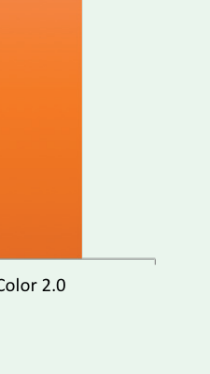
- Risk Free Storage
- No freezer breakdown
- Minimal disaster risks
- No increased HVAC costs

**Figure 5:** Many biobanks rely exclusively on freezer storage and this is not only acceptable for many samples it is an absolute requirement for tissue samples. The tissue cell matrix makes it impossible for the ACP matrix to quickly pass into the tissue samples to stabilize a tissue sample. It is much more appropriate to flash freeze tissue samples to stabilize them rapidly and for continued storage. However, freezer storage is subject to risks of sample loss caused by natural disasters that can interrupt electrical power for days, weeks or even months. McGill university did a survey to determine the risks involved in freezer storage and if the electrical issues caused loss of samples in the biobank. The results of this survey are shown here. The picture shown on the right is a photo of Manhattan after Hurricane Sandy that knocked out electrical power for so long that a biobank in the middle of the blacked out area had to be transferred to another storage site in another state to prevent sample losses.

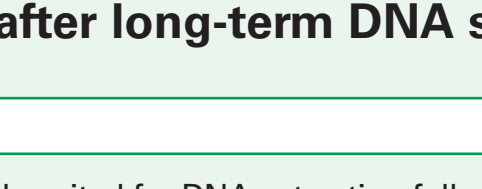
## GenSaver™ Collection Cards for Ambient Storage

Collection and storage of DNA and Biological samples

- GenTegra-DNA chemistry on card
- >20 Years of storage of whole blood, etc. at ambient
- 2x the DNA of FTA® Classic,
- 18x better long-term storage
- Direct PCR, no wash step

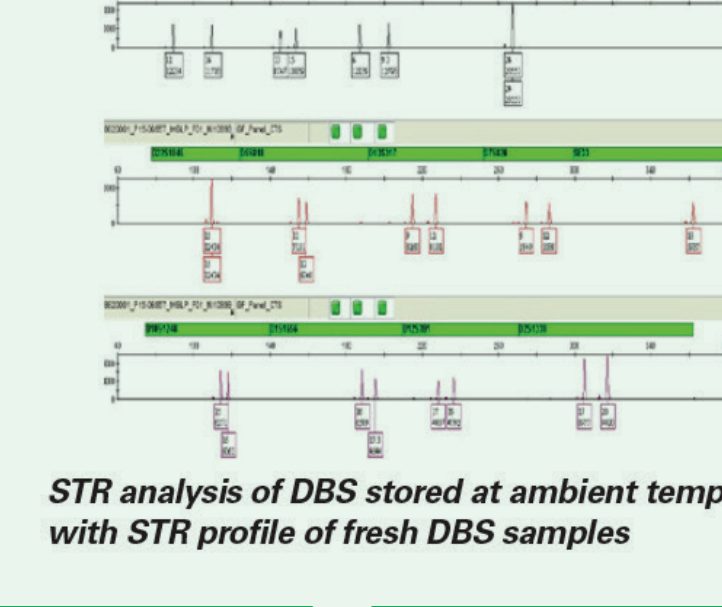


Biosample TFN cards: Protein card



**Figure 6:** The most common raw sample stored on collection cards is Blood and the DNA it contains. The GenSaver collection card is a new and superior replacement for the FTA Classic card. For the storage of proteins, the recommended collection card is a plain paper card which provides better protection for the proteins and eliminates any chemical interference with the Mass Spec analysis that is used for protein analysis. All GenSaver cards are manufactured by Ahlstrom-Munksjö under the ISO 18385 guidelines which ensure the cards are not contaminated with human DNA. The GenSaver 2.0 card is a version specifically designed for use in the forensic market and provides an antimicrobial component to stabilize the dirty samples often collected in forensic applications.

### DNA Stability Accelerated at 72°C

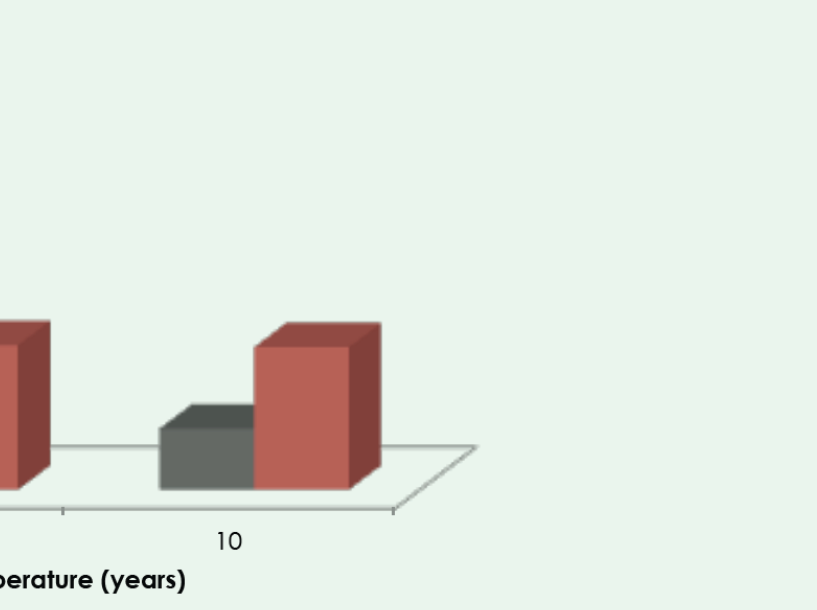


- GenCollect paper protects DNA 4x better than FTA Classic
- GenSaver paper protects DNA 18x better than FTA Classic
- GenSaver paper protects DNA 4x better than plain paper

**Figure 7:** An accelerated temperature storage comparison shows the results for blood DNA on FTA, GenCollect and GenSaver papers. The GenSaver paper with ACP protects the DNA 18 times better than FTA paper and the GenCollect card protect the DNA 4 times better than FTA paper. The FTA cards contain chemicals to cause lysis which are potentially harmful to the DNA.

## Next Generation Sequencing data

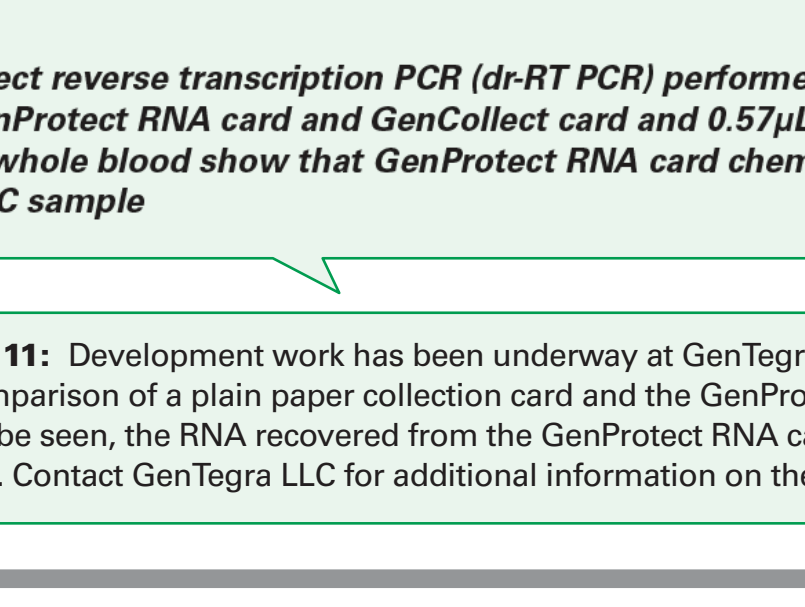
Ahlstrom-Munksjö cards	Years of storage	Bases	≥ Q20	Reads	Mean Read Length
GenSaver™ 2.0	15	35 899 842	34 420 724 (95%)	439 855	82 bp
	20	37 970 318	35 669 237 (95%)	472 610	80 bp
GenSaver™ Color 2.0	15	26 613 871	24 621 019 (90%)	325 937	79 bp
	20	38 027 859	35 626 196 (94%)	490 408	78 bp



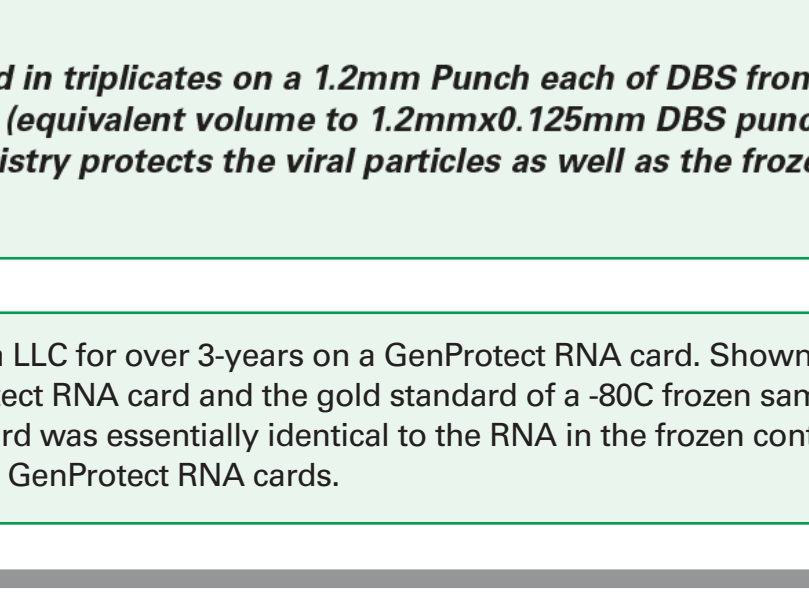
➔ NGS with high quality data achievable after long-term DNA storage

**Figure 8:** The chemically enhanced GenSaver collection cards are ideally suited for DNA extraction followed by NGS. The extracted and purified DNA is immediately ready for library prep and NGS analysis. This data shows excellent sequencing data for age accelerated samples of 15 years and 20 years and that the color version of the GenSaver 2.0 allows sequencing just as well as the GenSaver without the added color dye. The color dye allows use of cards for biological fluids that are colorless. The pink/purple dye goes white when an aqueous solution is added to the paper.

### GenSaver DBS at time Zero

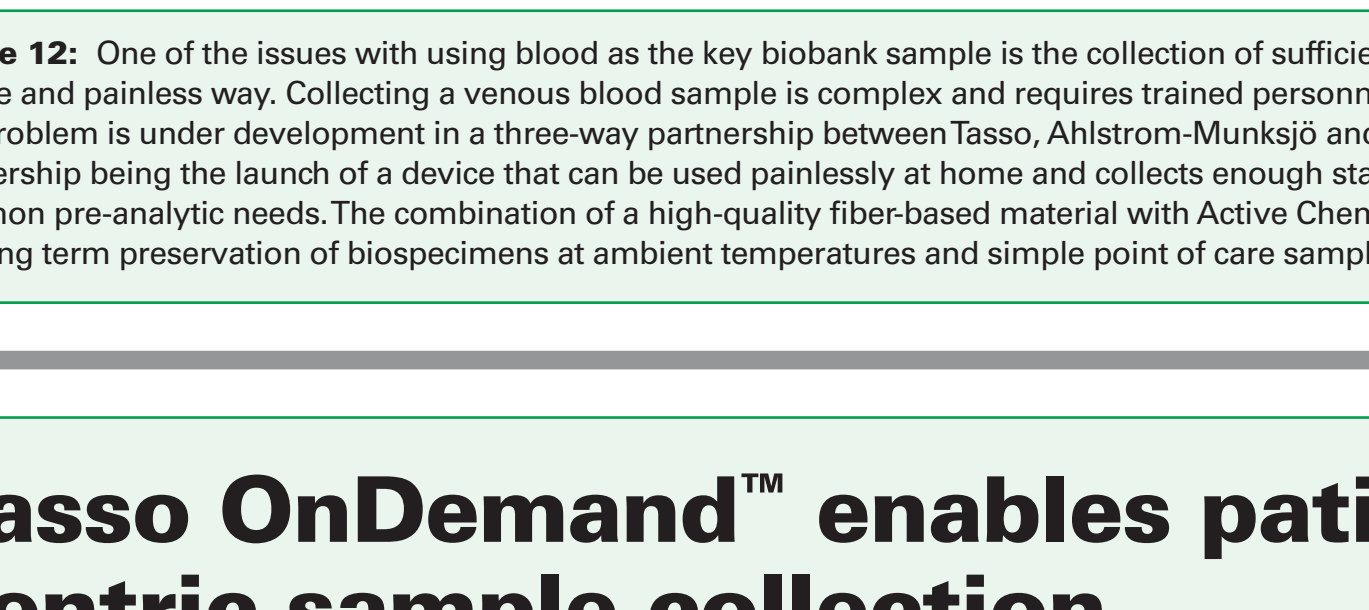


### GenSaver DBS at time = 2.5 years



STR analysis of DBS stored at ambient temperature for 2.5 years (accelerated study) show 100% concordance with STR profile of fresh DBS samples

**Figure 9:** The dominant DNA technology used in forensics is STR technology to provide HID results for identification of forensic DNA samples. This figure shows a common output for and STR analysis of a HID sample. The report on the left is a STR analysis for a fresh DNA sample and the report on the right is the same sample after temperature acceleration to age of 2.5 years. As the STR technology has evolved over the past decades the typical HID analysis has added markers and is now at 20 for the FBI CODIS database. Prior to 1998 the core loci were 13. As the markers have increased in number, they have also been shortened to make the HID analysis better able to handle older degraded DNA samples. This data shows complete concordance between the fresh DNA sample and the 2.5 years old sample.



**Figure 10:** This data shows a temperature accelerated time curve comparing the yield of DNA from GenSaver & FTA paper at zero time, 5 years and 10 years. In all cases, the same standard extraction protocol using GenSolve was used for direct comparison. Time zero and times of up to a year show significantly more available DNA than older samples. The DNA available at 5-years and then 10 years is not really lost over time but is due to the increasing difficulty of extracting the DNA from the matrix of dried cell debris and surrounding protein complex. As mentioned, the same GenSolve extraction protocol was used for this data. However, we know that to optimize the amount of DNA extracted for older DBS much longer incubation of the DBS sample in the lysis solution is required. If the DBS punch is incubated overnight in the GenSolve lysis buffer the DNA yield goes up from two to ten-fold. A good rule would be to incubate all DBS samples 5-year or older in lysis solution overnight to optimize DNA yield. This modified extraction data is not shown.



Direct reverse transcription PCR (dr-RT PCR) performed in triplicates on a 1.2mm Punch each of DBS from GenProtect RNA card and GenCollect card and 0.57µL (equivalent volume to 1.2mmx0.125mm DBS punch) of whole blood show that GenProtect RNA card chemistry protects the viral particles as well as the frozen -80°C sample

**Figure 11:** Development work has been underway at GenTegra LLC for over 3-years on a GenProtect RNA card. Shown here is a comparison of a plain paper collection card and the GenProtect RNA card and the gold standard of a -80°C frozen sample. As can be seen, the RNA recovered from the GenProtect RNA card was essentially identical to the RNA in the frozen control sample. Contact GenTegra LLC for additional information on the GenProtect RNA cards.

## Complete Solution for painless collection of DBS from collaboration between Ahlstrom-Munksjö and GenTegra integrated with Tasso On-Demand™ device

AHLSTROM MUNKSJÖ  
High quality fiber-based material fibers

Treatment technologies  
Converting

GenTegra  
Active Chemical Protection™

Nucleic acids preservation at ambient temperature

Tasso OnDemand™

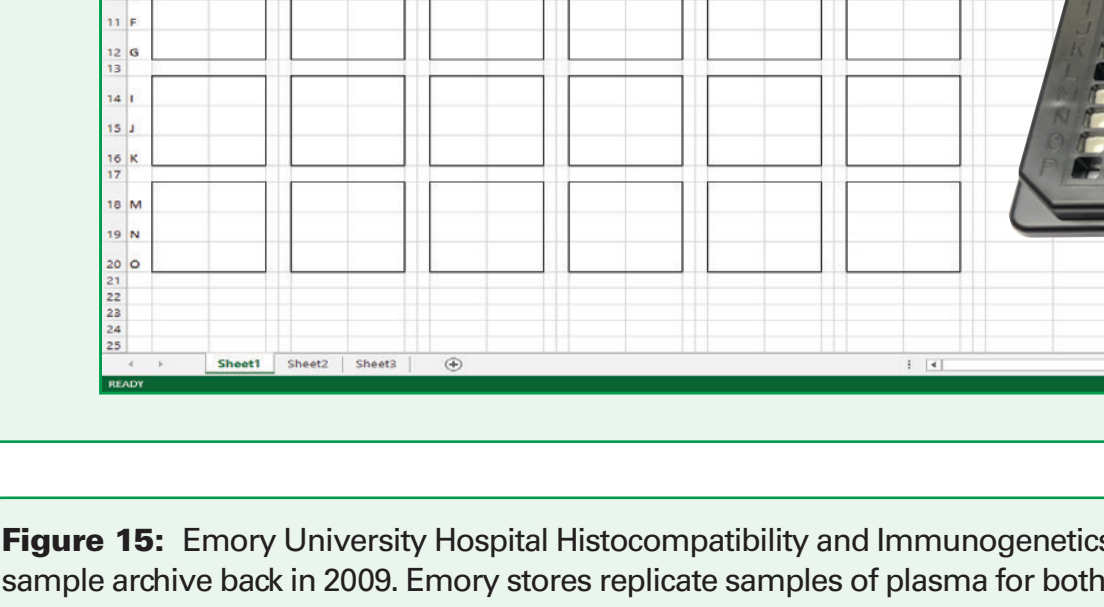
Painless collection of Whole blood sample on GenSaver

GenTegra LLC and Ahlstrom-Munksjö GenSaver™ Card can be integrated in the Tasso OnDemand device for painless extraction of up to 200µL of whole blood on GenSaver card.

Tasso OnDemand™: Less Pain; Volume control ±5%; Stabilization with GenProtect II

**Figure 12:** One of the issues with using blood as the key biobank sample is the collection of sufficient blood volume in a simple and painless way. Collecting a venous blood sample is complex and requires trained personnel. One potential answer to this problem is under development in a three-way partnership between Tasso, Ahlstrom-Munksjö and GenTegra. The goal of this partnership being the launch of a device that can be used painlessly at home and collects enough stabilized blood for all common pre-analytic needs. The combination of a high-quality fiber-based material with Active Chemical Protection will allow the long term preservation of biopspecimens at ambient temperatures and simple point of care sample collection.

## Tasso OnDemand™ enables patient-centric sample collection



- Patient-centric solution
- Painless and convenient
- Samples at the press of a button
- At-home collection
- Higher patient satisfaction
- Replaces a lab visit
- High quality samples
- 4 DBS spots with exact volume, ±5%
- Stabilized analytes

**Figure 13:** The GenTegra, Ahlstrom-Munksjö partnership was extremely fruitful and lead to the development and commercialization of the line of GenSaver cards. Cards that outperform other cards in virtually every application and need for collection of DNA from biological samples. And Tasso has developed a collection device called "On-Demand" that makes taking a controlled volume sample of blood possible in a home setting. Maybe a bit more costly than using a card but the Tasso device adds a completely new level of volumetric collection in a home environment. And the device is much more convenient than going to a lab and much less expensive than requiring a phlebotomist.

## DBS and Biosample Products

GenPlates

Long-term, high density storage of blood, blood products and biosamples

GenSolve

High yield DNA extraction of biosamples from GenSaver and filter papers

Archive Cabinets

High density convenient GenPlate storage at ambient temperature



## Brief Case Study for using paper collection and automation friendly GenPlates for archive support of an HLA laboratory



**Figure 15:** Emory University Hospital Histocompatibility and Immunogenetics Lab adopted the GenPlate as the basis for their HLA sample archive back in 2009. Emory stores replicate samples of plasma for both the organ and the patient receiving the transplant. There is a semi-automated approach where they use humidity-controlled Personal Archive cabinets specifically designed for GenPlate storage and a Sample tracking program. To assist accurate sample placement into the GenPlates they use a working EXCEL spreadsheet designed at Emory that visually matches the layout of their GenPlate. Later the data from the spreadsheet is transferred into their sample tracking software. The Personal Archive storage cabinet provides a completely barcoded system for each and every storage location of the GenPlates in the cabinets and each GenPlate has its own unique barcode.